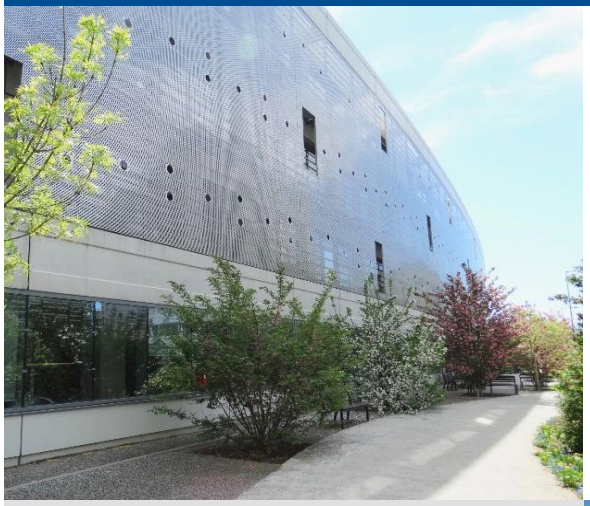
Environment

Analysis of micropollutants in solid matrices: from broad screening to quantification



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Commission européenne

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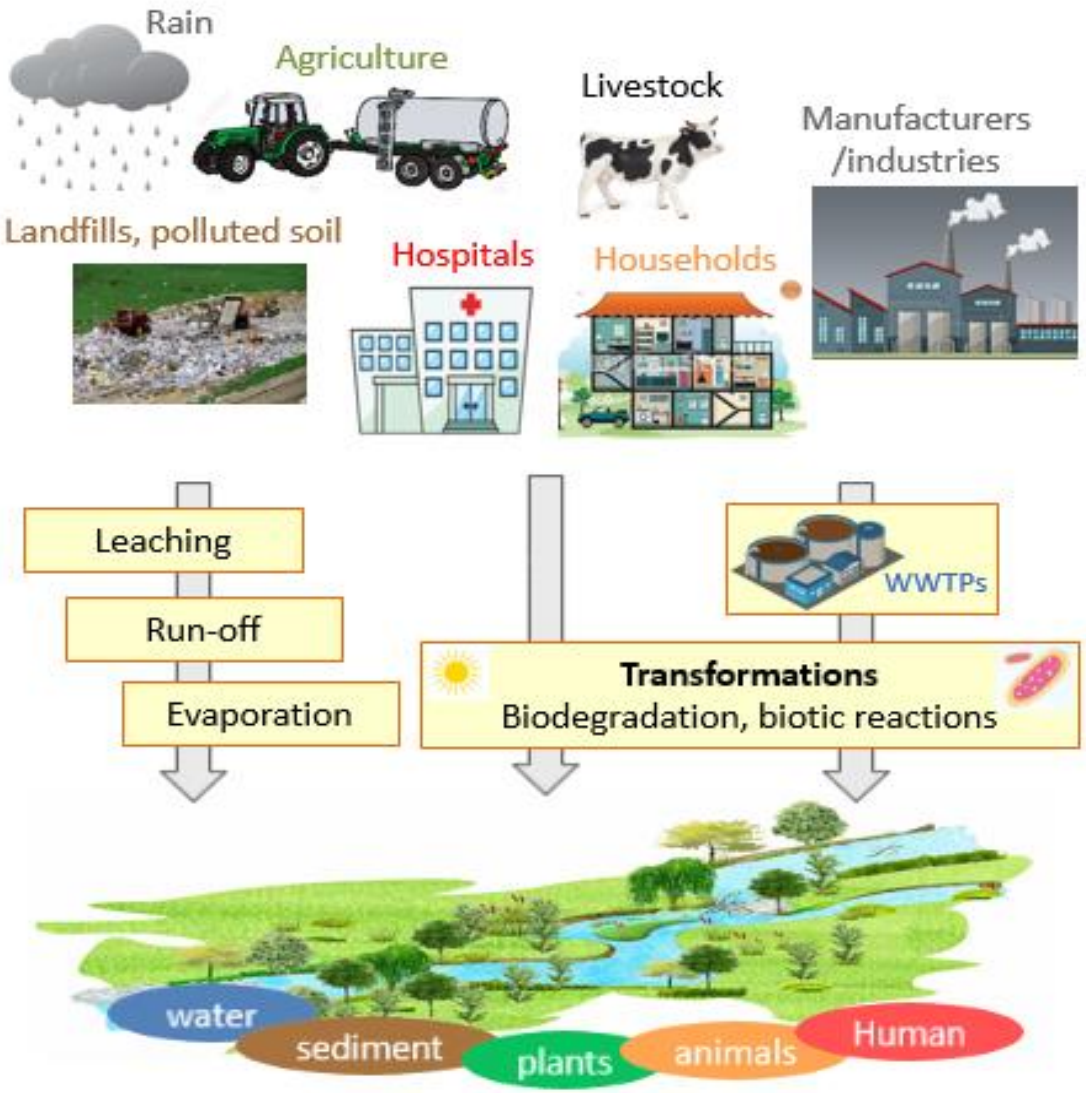
Micropollutants in the environment

Anthropogenic activities responsible for widespread environmental contamination

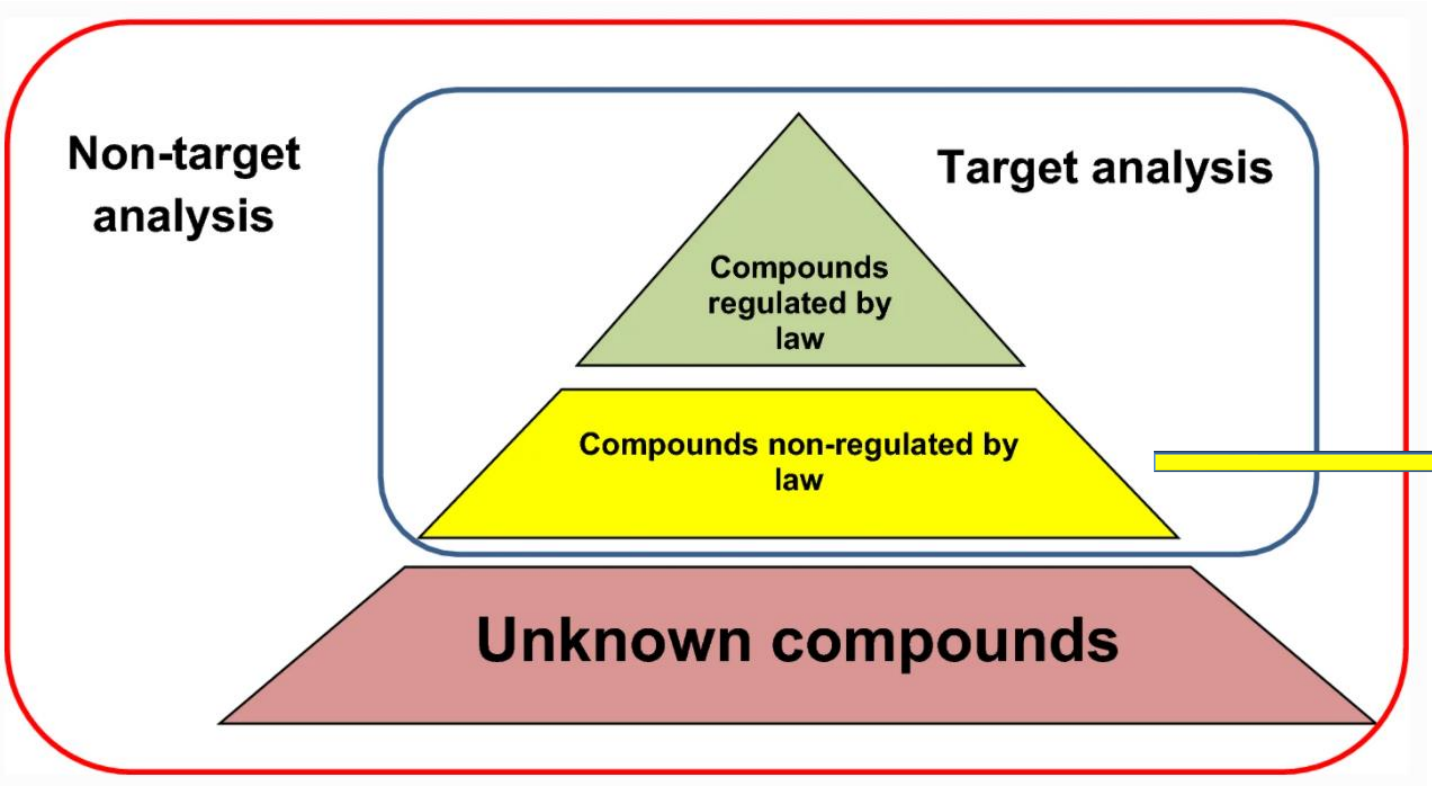
sources of micropollutants

transport and transformation

receiving environment



Analysis of micropollutants in the environment



The so-called

« Emerging » contaminants (EC)

or

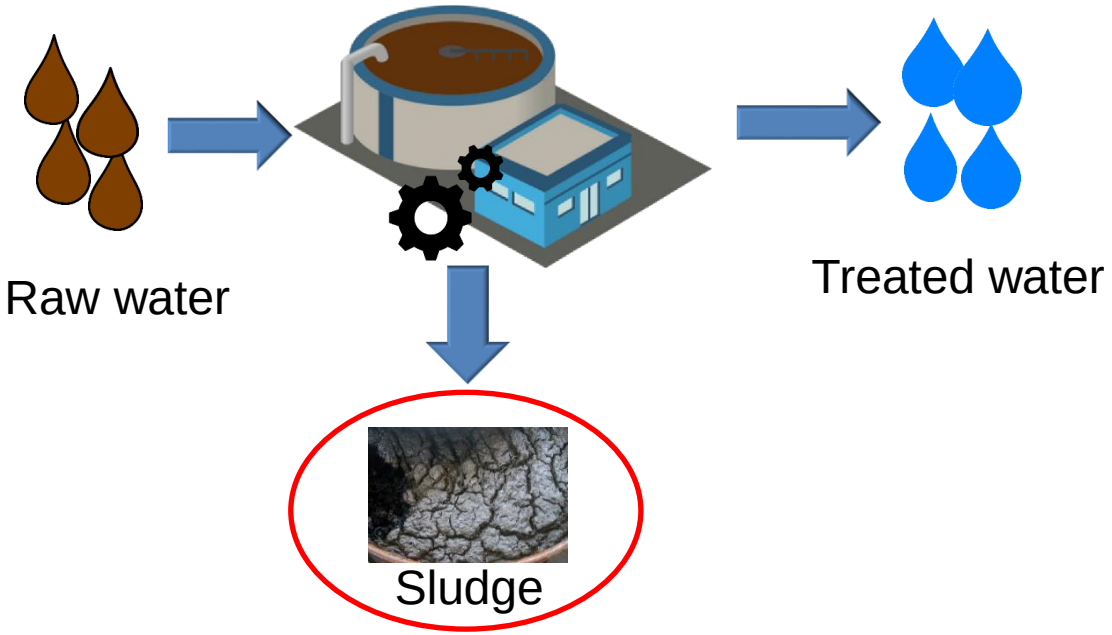
Contaminants of Emerging Concern (CEC)

Rogowska J et al. Ambio 49 (2020) 487-503

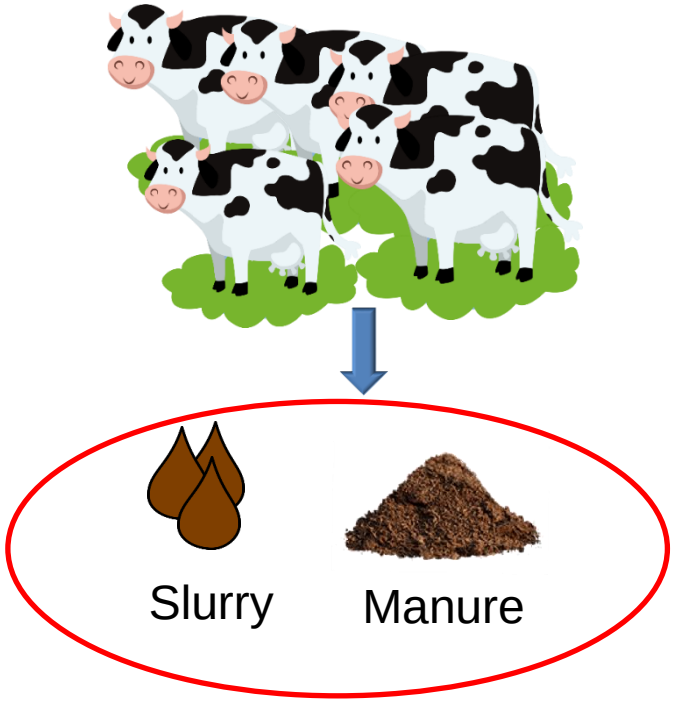
- Antibiotic issues
- Extraction of beta-lactam antibiotics
- Advantages of MRM^3 vs MRM mode
- Suspect screening

Environmental solid matrices

Wastewater treatment



Livestock manure



SOLID MATRICES
Organic wastes such as sewage sludge and manure:
Exogenous organic matters (EOMs)

Environmental solid matrices: problematic

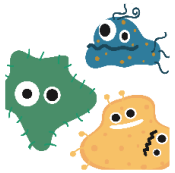
EOM recycled in agriculture for their agronomic potential

Livestock manure

- 99% agricultural
- 1% thermal

Sludge

- 73% agricultural
- 18% thermal
- 9% landfill



Antibiotic resistance

Bioaccumulation

Leaching
Transfer

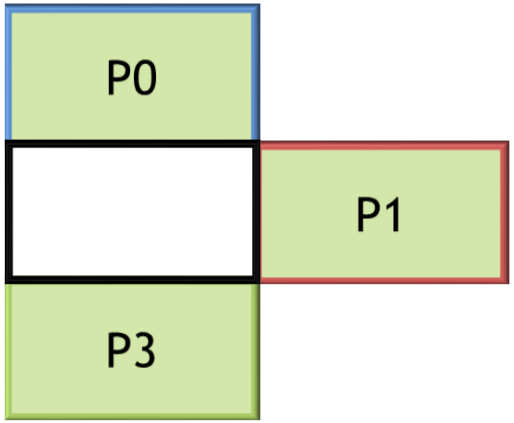
Use of antibiotics in human or veterinary medicine:

- Poorly assimilated
- Rejected in EOM

Experimental site

RISMEAU SIPIBEL Site Pilote de Bellecombe

Study carried out on 3 experimental plots on the same site

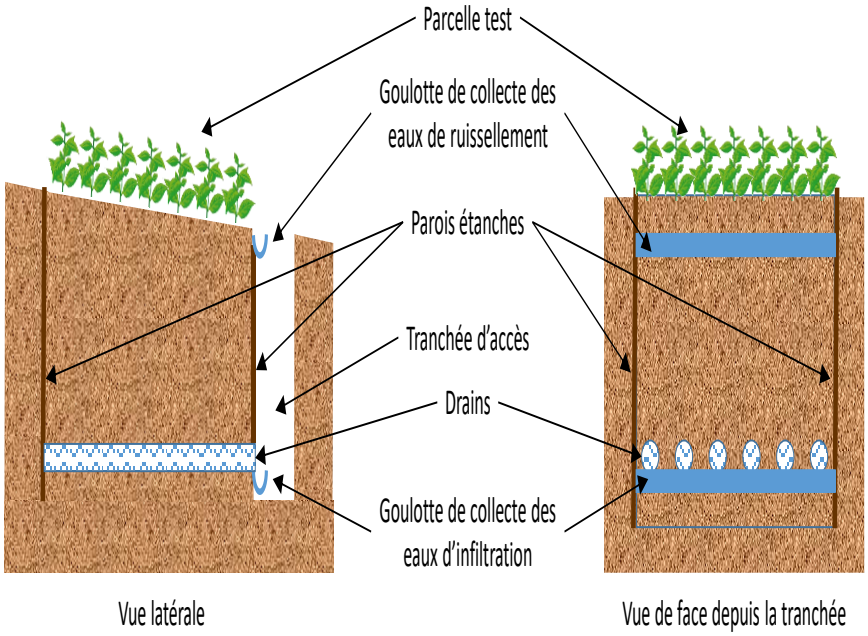


Study carried out on 3 experimental plots on the same site:

- soil without input
- soil after spreading of WWTP sludge
- Soil after spreading of agricultural inputs (slurry or cattle manure)

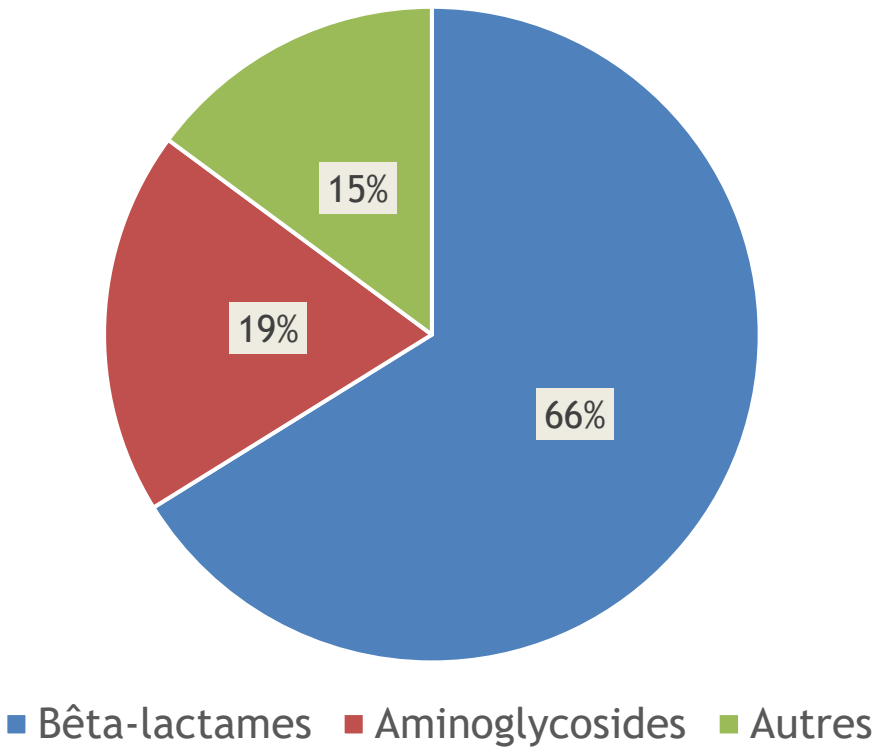
Sampling:

- Infiltrated water
- Spreading EOMs
- Soil columns



Which contaminants to monitor ?

Analysis of the sanitation diary of 5 farmers over a period of 3 years



Two main families:

Beta-lactams

Aminoglycosides

Analytical challenges throughout the protocol

Extraction

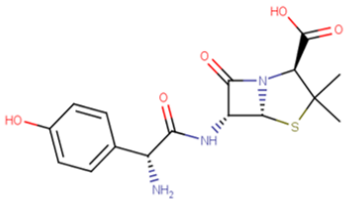
- Heterogeneous environmental matrices
- Absence of reference matrix
- Instability of beta-lactams

Separation

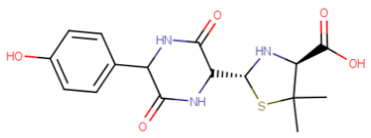
Detection

- Antibiotic issues
- **Extraction of beta-lactam antibiotics**
- Advantages of MRM^3 vs MRM mode
- Suspect screening

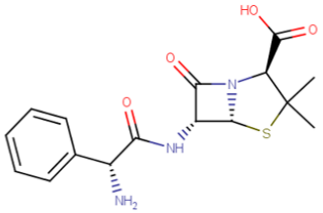
Targeted beta-lactams



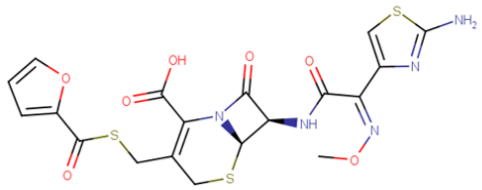
Amoxicillin (AMX)



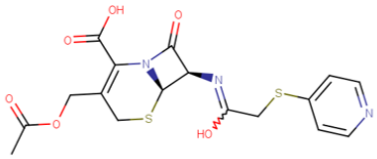
Amoxicillin diketopiperazine (AMX-DKP)



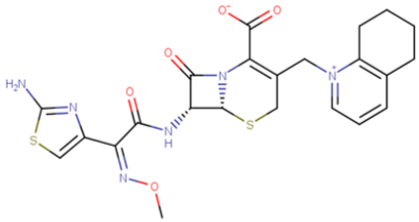
Ampicillin (AMP)



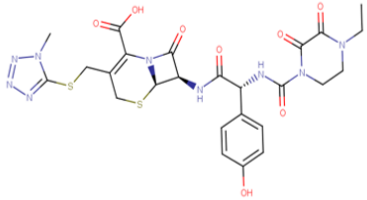
Ceftiofur (CEF)



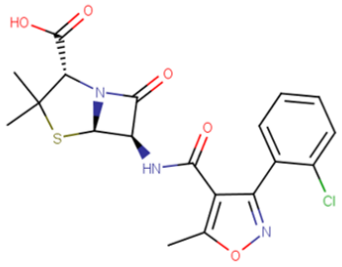
Cefapirine (CFP)



Cefquinome (CFQ)



Cefoperazone (CFZ)

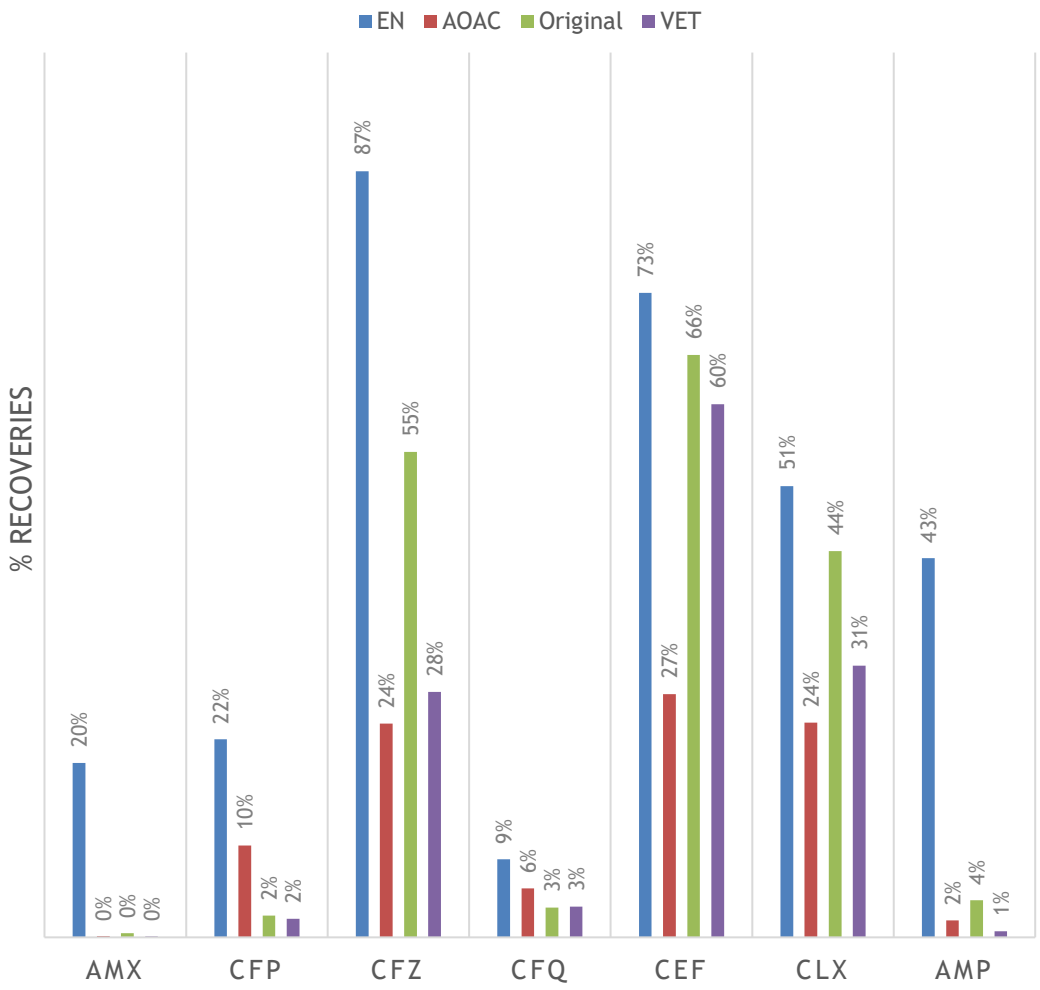


Cloxacillin (CLX)

7 parent molecules and one metabolite

Development of the extraction method

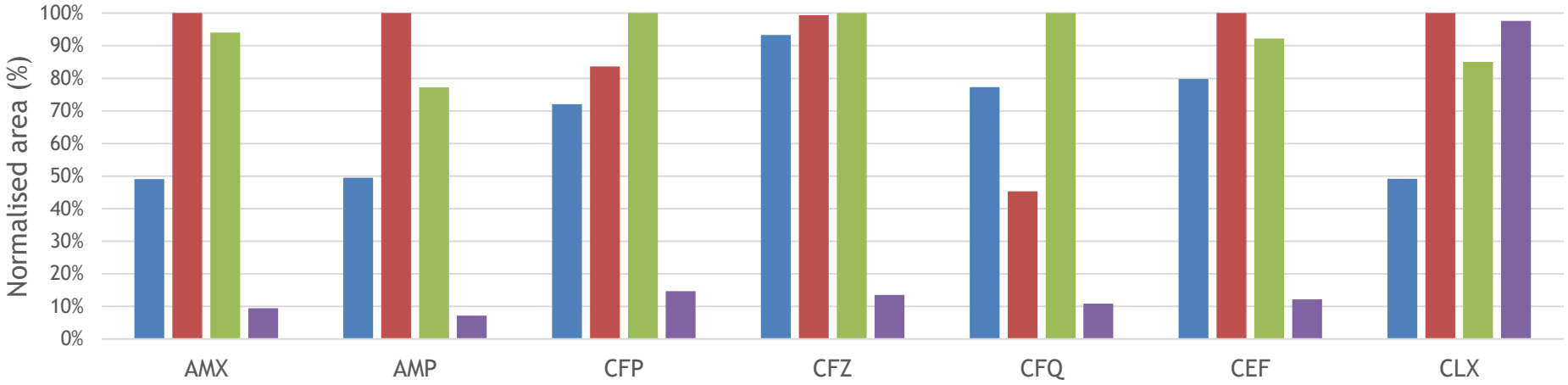
QuEChERS: Testing of different extraction kits (EN, AOAC, Original, Veterinary Drugs)



EN method (citrate) : only one that allows the extraction of amoxicillin + best recovery on all molecules

- Lowers the pH of the extract and decreases the polarity of the analytes resulting in a better transfer into the organic phase
- Contains citrate salts chelating metal cations => better extraction of organic compounds

Development of the extraction method



Various aqueous phases

Eau / Eau + EDTA 0.1M / Eau + Formic acid (FA) 0.1% / Eau + EDTA 0.1 M + FA 0.1%

- ✓ Addition of EDTA : Chelation of metal cations=> release of organic cations
- ✓ Addition of formic acid: Reduction of the negative surface charge of the sample – release of matrix bound species
- × Combinaison of both: Acid modifies the ionisation of EDTA which becomes monoanionic (H3Y-) – decrease of its chelatig power

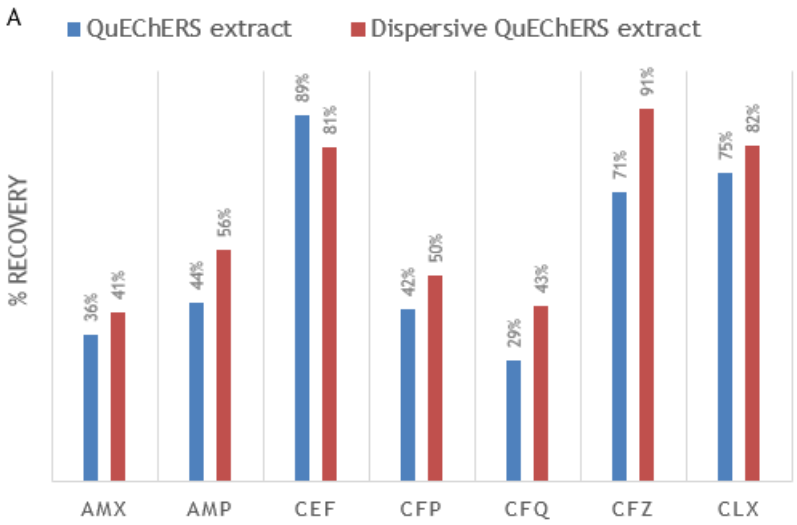
Development of the extraction method

Addition of a « matrix dispersion » step: increases the contact surface of the sample with the solvent

EDTA-treated Fontainebleau sand used to promote dispersion

Extraction recovery

Matrix effect



Development of the extraction method

Extraction QuEChERS

Acetate	Citrate	Original	Veterinary compounds

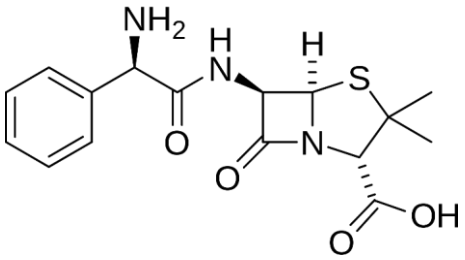
SPE dispersive purification

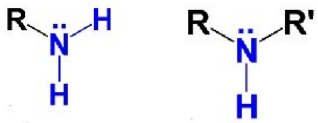
Addition of $MgSO_4$: drying the organic phase

Primary Secondary Amines (PSA)	PSA/C18

Beta-lactams, <5% recovery after dispersive SPE

H-bonding between COOH and amines





Other option: purification on SPE cartridge

1/ Conditioning: ACN

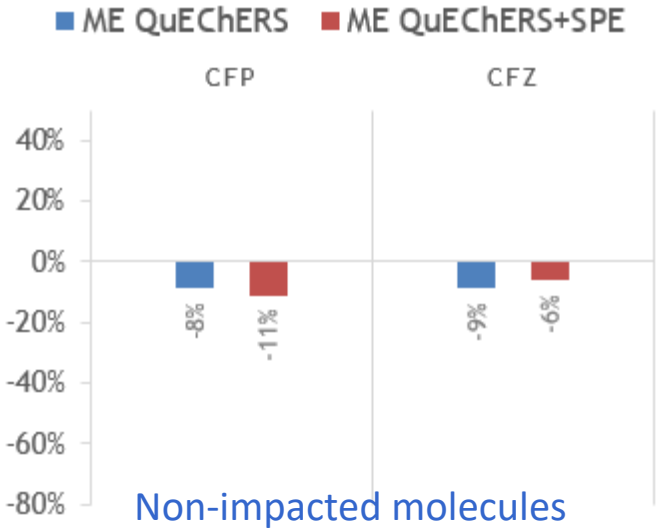
2/ Purification

QuEChERS extract

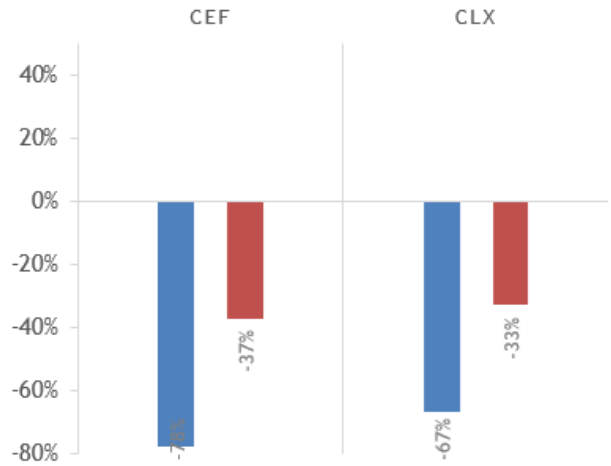
Purified extract

Development of the extraction method

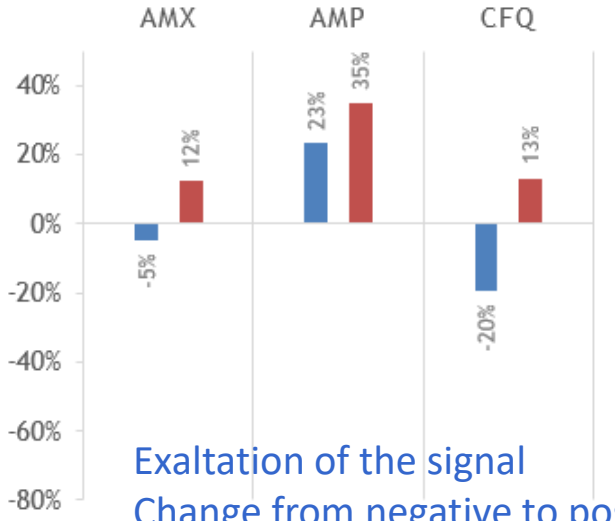
Modification of matrix effects (ME): 3 cases



Non-impacted molecules



Reduction of ME

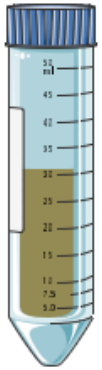


Exaltation of the signal
Change from negative to positive ME

Final condition of extraction

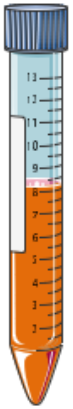
Sample dispersion
- 500 mg sample
- 2 g sand

QuEChERS extraction
- H_2O + EDTA 0.1 M
- ACN
- EN buffer (citrate)



Supernatant recuperation
- Centrifugation
- Transfer

Purification
- Direct SPE on StrataX

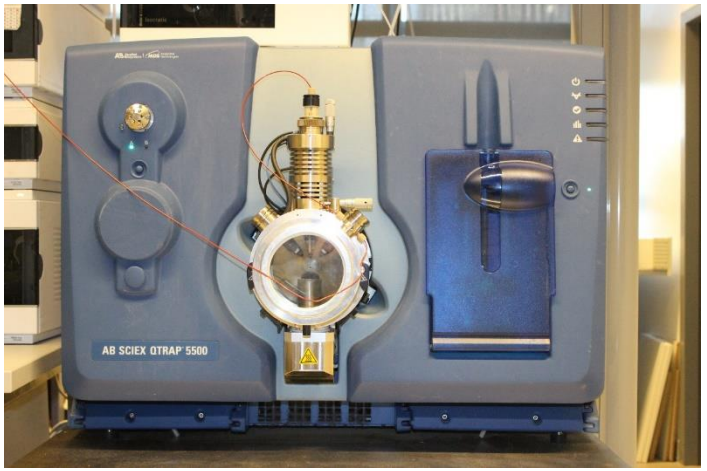


Analytical conditions

Separation : UPLC 1290 Infinity, Agilent

Column : Kinetex F5 , 100 x 2.1mm, 1.7 µm (Phenomenex)
A: H_2O + 0.1% Formic Acid
B: ACN + 0.1% Formic Acid

Detection : 5500QTrap, AB Sciex

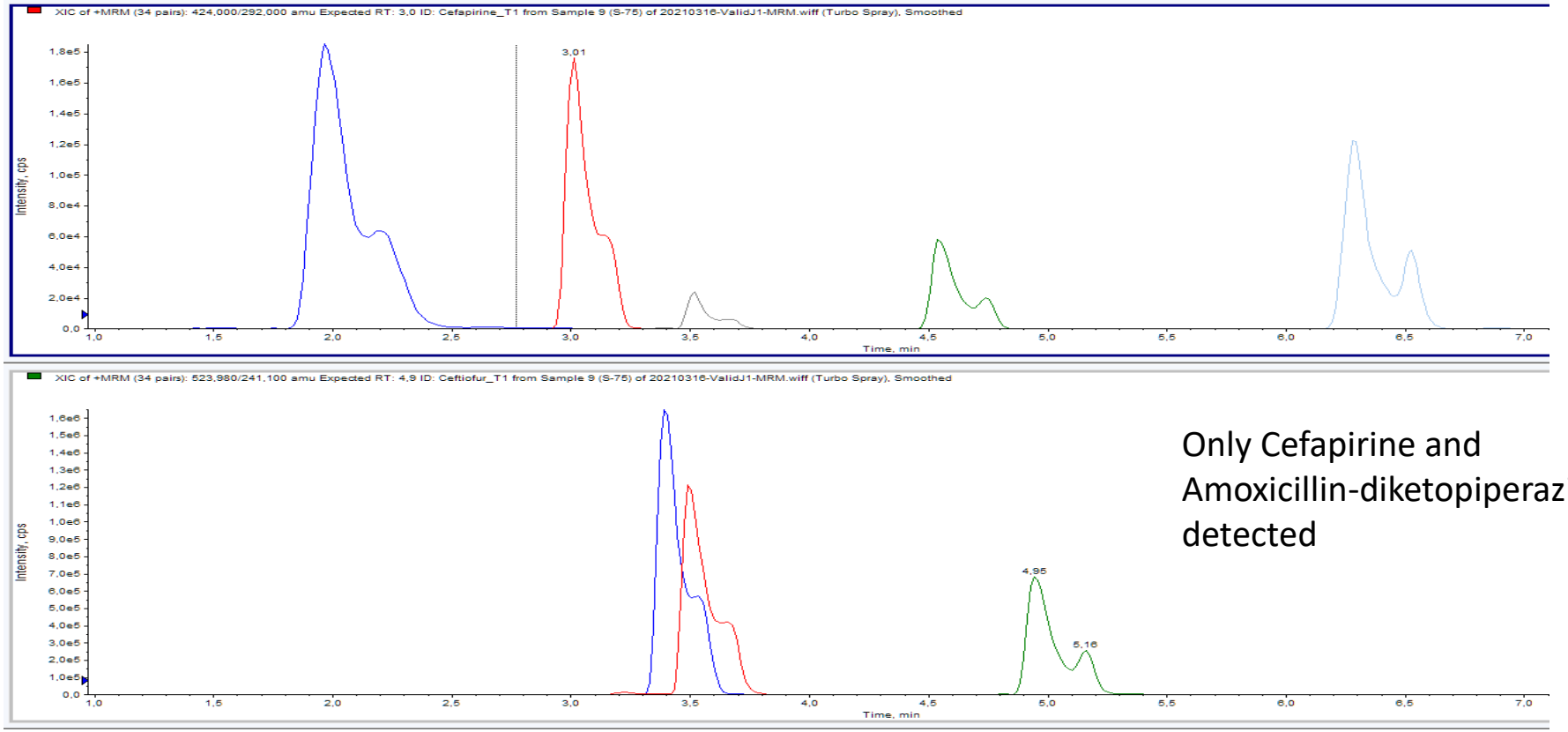


Qtrap are QqQ mass spectrometers with a linear trap for Q3

Application

Analysis of sludge collected over a 3 year period

Molecule	Detection frequency	C_{min} (ng/g)	C_{moy} (ng/g)	C_{max} (ng/g)
CFP	50%	10.2	23.5	53.3
AMX-DKP	50%	3.0	5.3	9.5



Only Cefapirine and Amoxicillin-diketopiperazine detected

Analytical challenges throughout the protocol

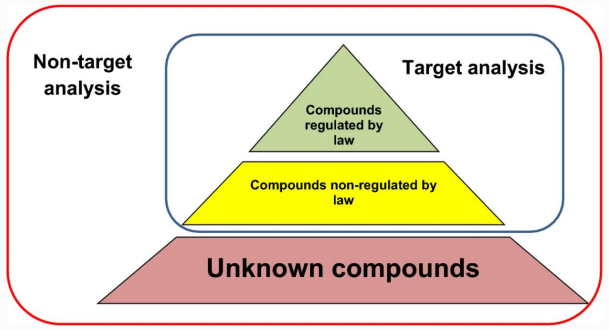
Extraction

- Heterogeneous environmental matrices
- Absence of reference matrix
- Instability of beta-lactams

Separation

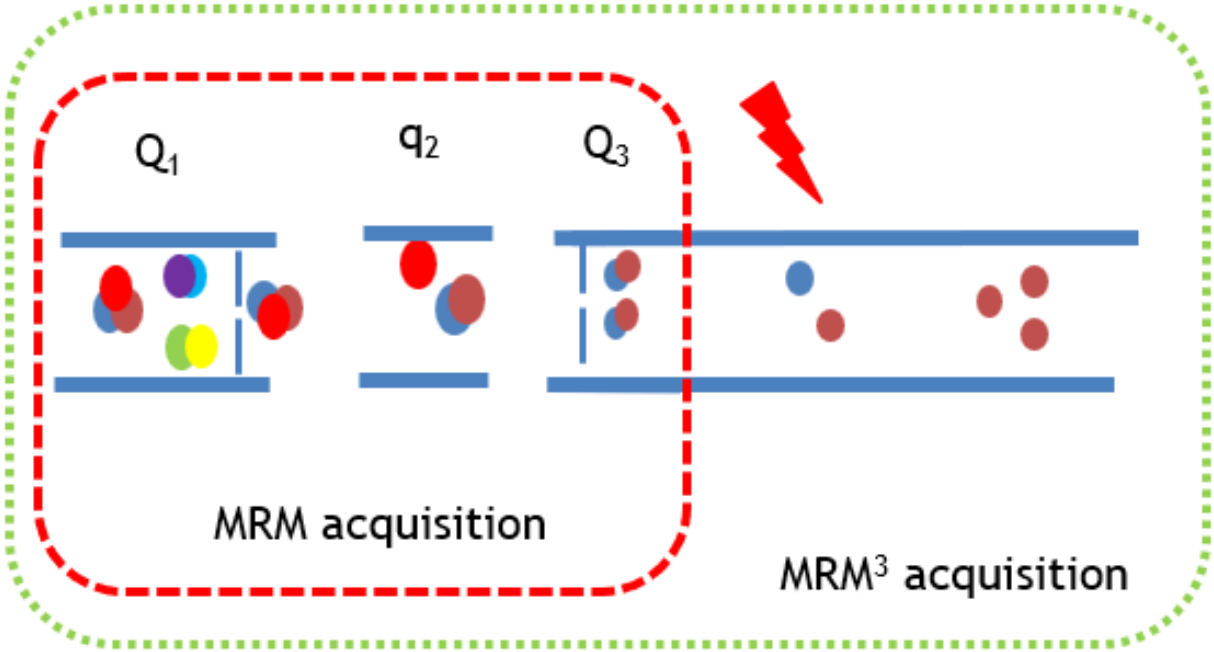
Detection

- Compounds present at traces levels
- Expending the number of compounds analysed

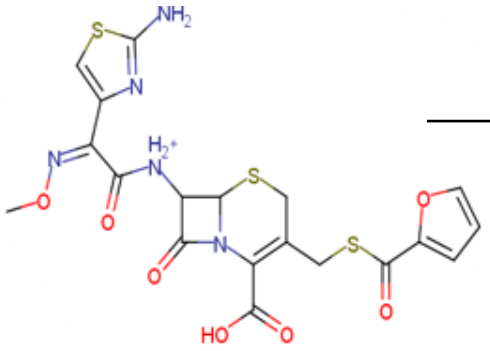


- Antibiotic issues
- Extraction of beta-lactam antibiotics
- Advantages of MRM^3 vs MRM mode
- Suspect screening

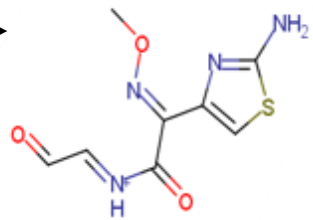
How to increase sensitivity: MRM *vs* MRM^3



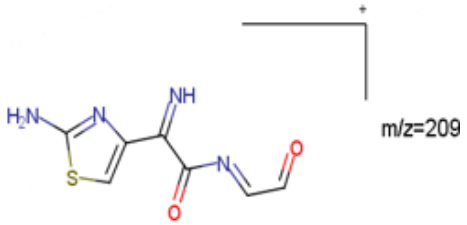
MRM: MS/MS
MRM^3: MS/MS/MS



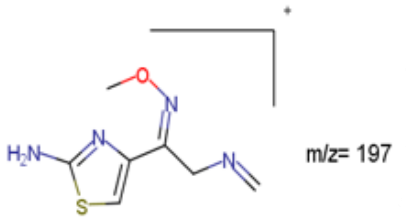
Ceftiofur
$C_{19}H_{17}N_5O_7S_3$
[M+H]+ m/z= 524



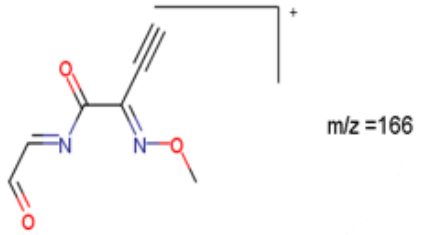
m/z=241
$C_8H_9N_4O_3S$



m/z=209

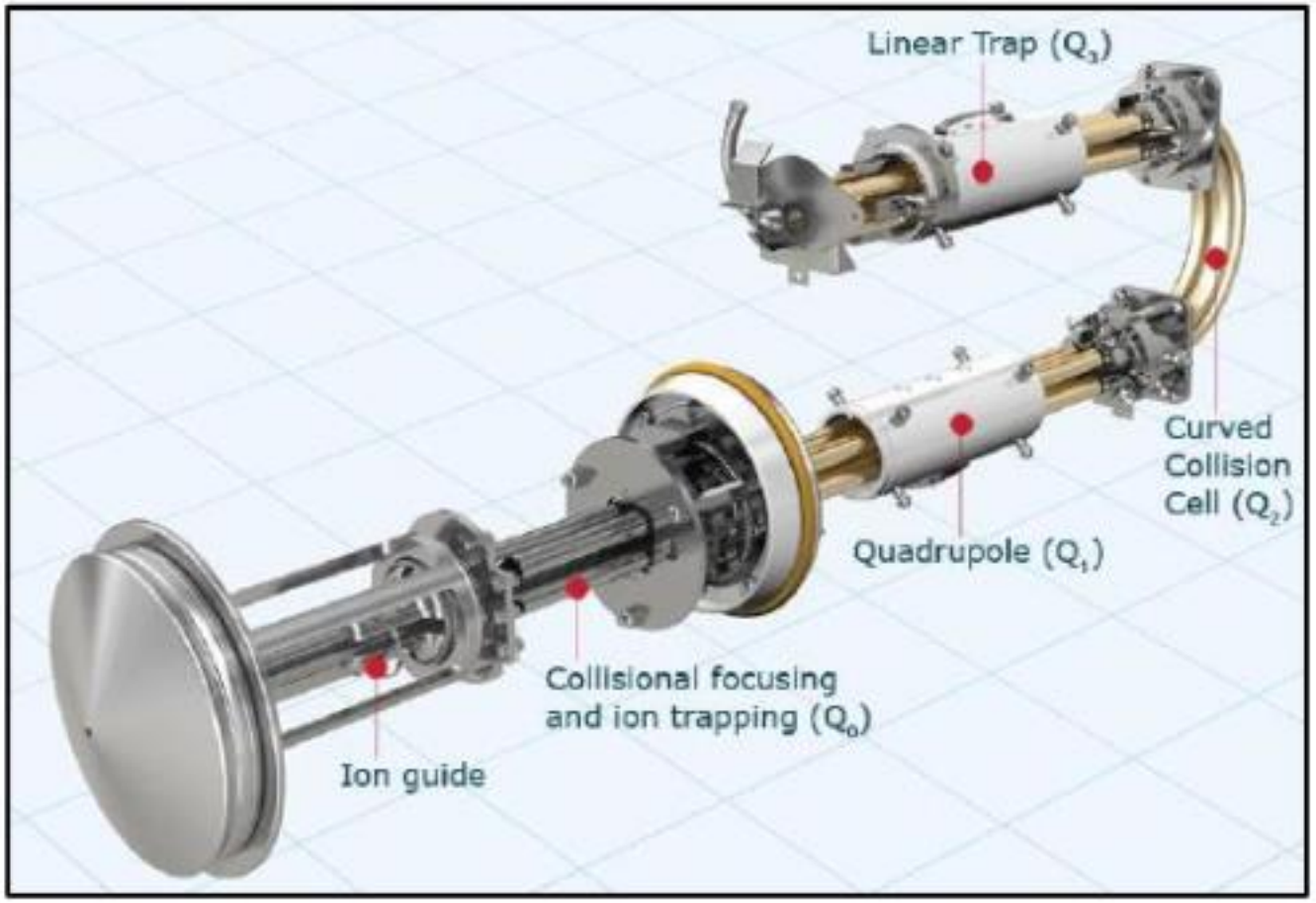


m/z= 197



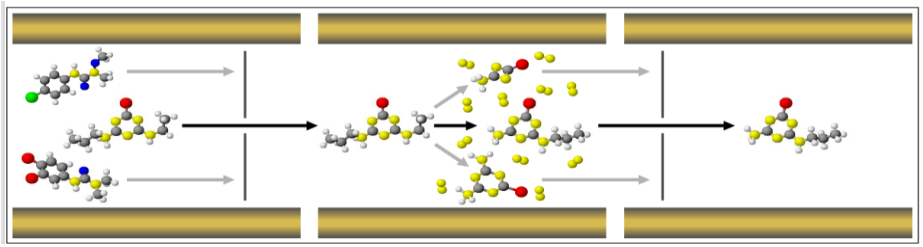
m/z =166

QTrap - Mode MRM^3

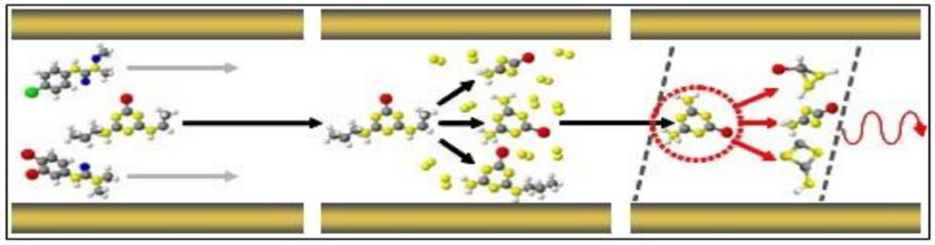


Optimization of the acquisition in MRM^3

MRM vs MRM^3



Transition P1 → F1



Transition P1 → F1 → F1’

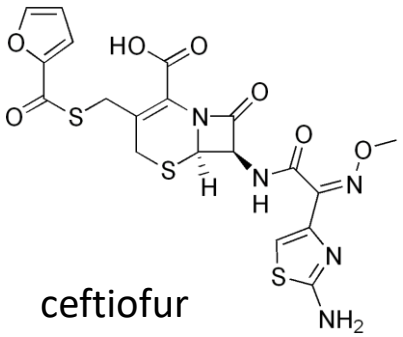
MRM parameters:

- Declustering potential (DP)
- Collision cell entrance and exit potentials (EP, CXP)
- Collision energy (CE)

Specific parameters MRM^3

- Q3 excitation energy (AF2)
- AF2 application time (Ex Time)
- Accumulation time in Q3 (LIT time)
- Q0 ion focalization

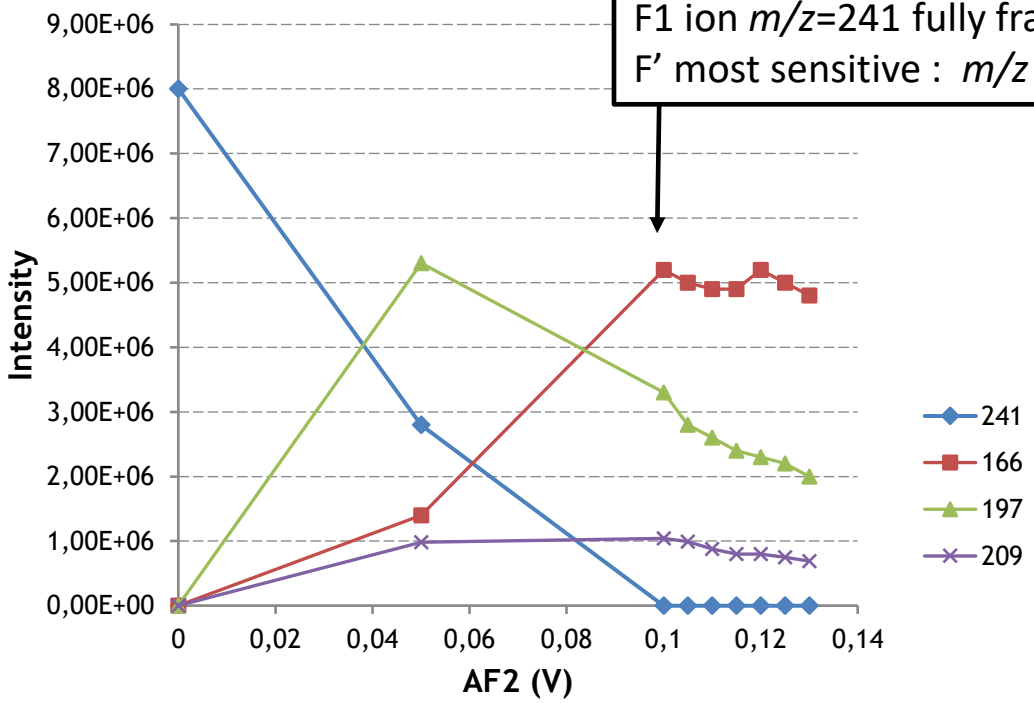
Example of ceftiofur (524 → 241 → 166)



ceftiofur

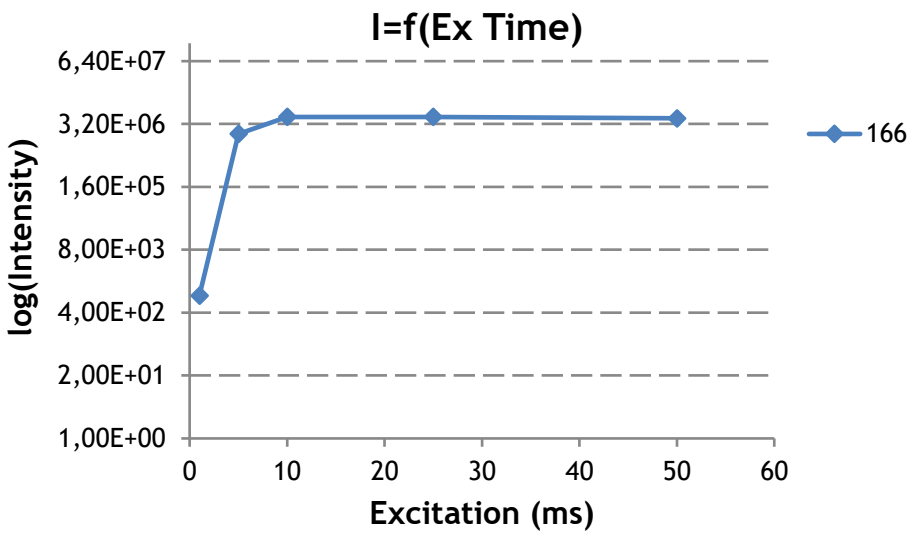
Variation of fragmentation energy

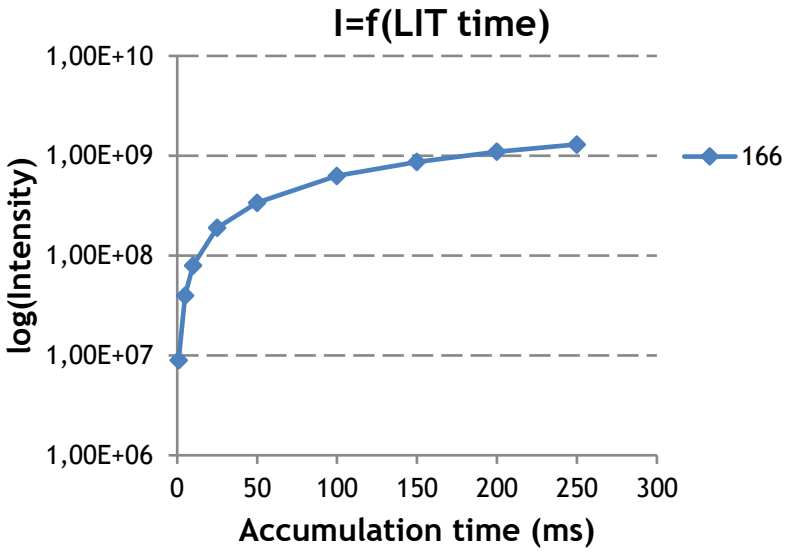
AF2 = 0.1:
F1 ion *m/z*=241 fully fragmented
F' most sensitive : *m/z* =166



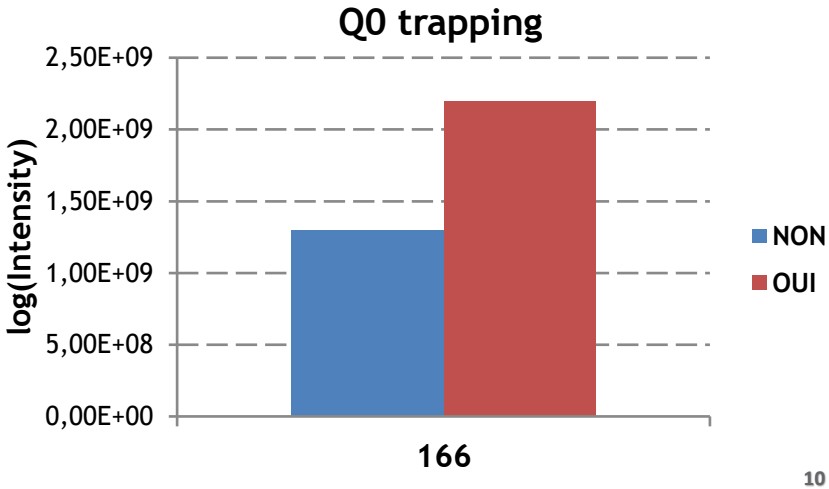
Example of ceftiofur (524 → 241 → 166)

Transition 524 → 241 → 166



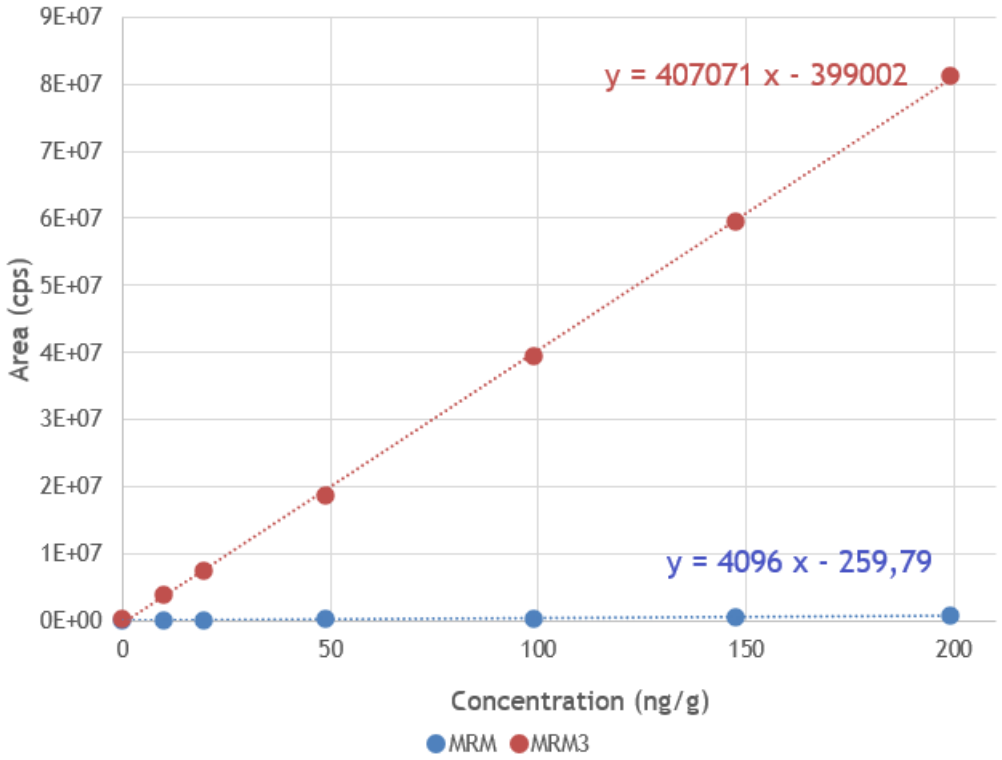


- After 25 ms excitation , no intensity increase of *m/z*=166: F1 ion is fully fragmented
- Up to 100 ms of accumulation, less than 10% intensity increase
- Better focalization allows up to 100% signal increase



Comparison with LC-MS/MS

1st interest: sensitivity

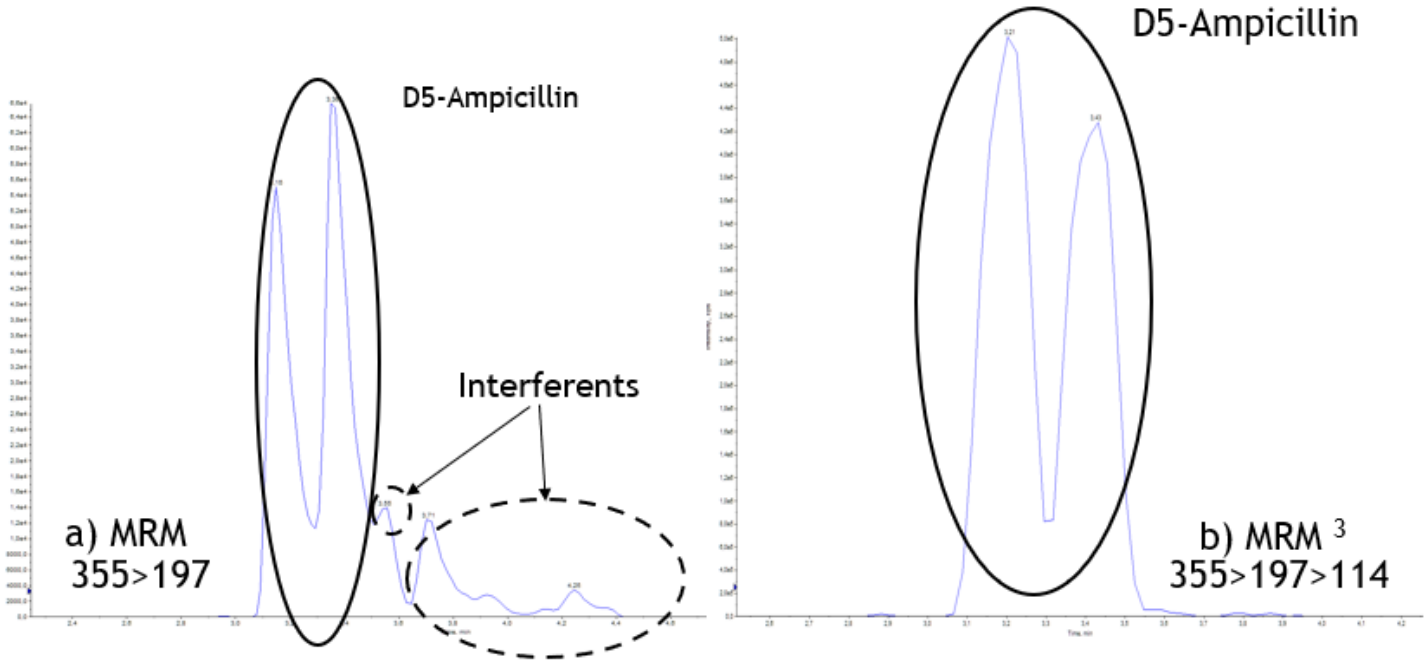


MRM^3 acquisition provides better sensitivity:

- ✓ Slope of the calibration curve is 100 times higher compared to MRM acquisition.
- ✓ Detection of lower concentration variation between samples
- ✓ Better absolute quantification precision

Comparison with LC-MS/MS

2nd interest: selectivity

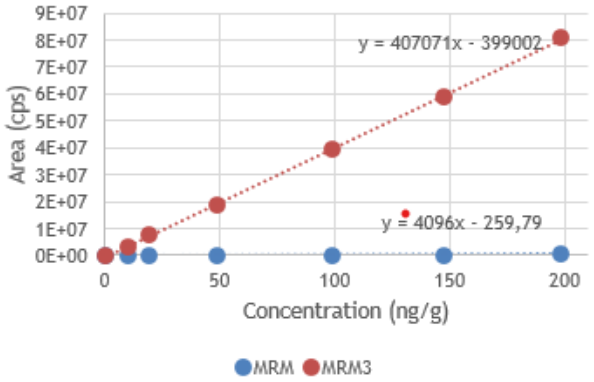


Adding an extra level of specificity with the second generation transition

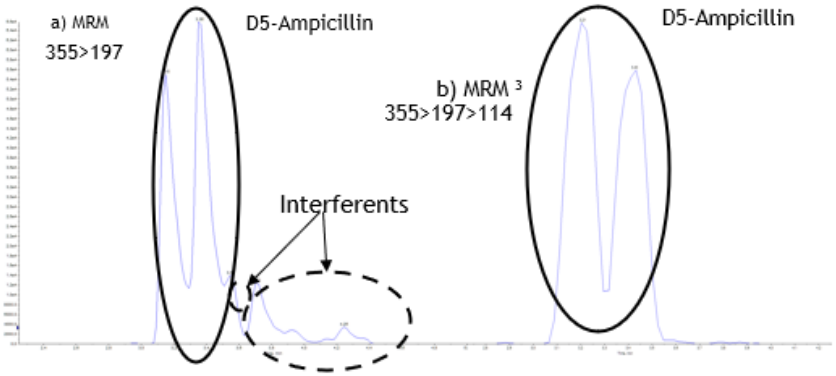
Removal of shouldering and interfering peaks

Comparison with LC-MS/MS

1st interest : Sensitivity



2nd interest : Specificity



Impacts on the limits of quantification (ng/g)

Increasing the limits

	MRM³	MRM
CEF	0.8	1.9
AMX	11.9	17.4
CFP	3.8	8.7

Lowering the limits

	MRM³	MRM
AMP	5.9	2.4
CLX	14.8	7.7

Hypothesis

Low mass daughter ion (<200 Da) + low loss (46 Da)
=> increased transition background

- Antibiotic issues
- Extraction of beta-lactam antibiotics
- Advantages of MRM^3 vs MRM mode
- Suspect screening

Interest of suspect screening

Lists of molecules for targeted analysis

- Not relevant to the project
- Availability of analytical standards

New list

- More representative of the sample
- More suitable extraction and analysis methods

Suspect screening

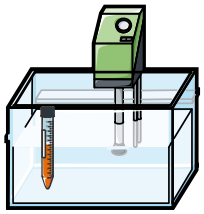
- Comparison to databases
- Detection of compounds present in the matrix
- Identification of a wider variety of molecules

Confirmation

- Standards only for suspected molecules

Suspect screening

Strategy on different matrices using databases:



Soil

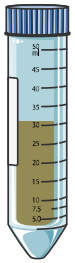
Sludge

Slurry

Manure

Extraction 1:
Ultrasonication
H_2O/Methanol

Extraction 2:
QuEChERS AOAC

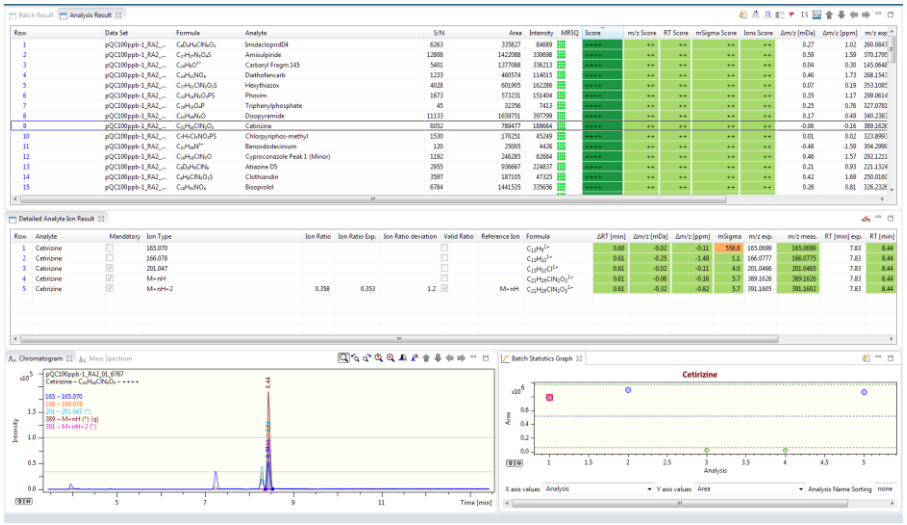


Analysis 1: LC-QqQ
Quanpedia (Waters)
160 veterinary drugs

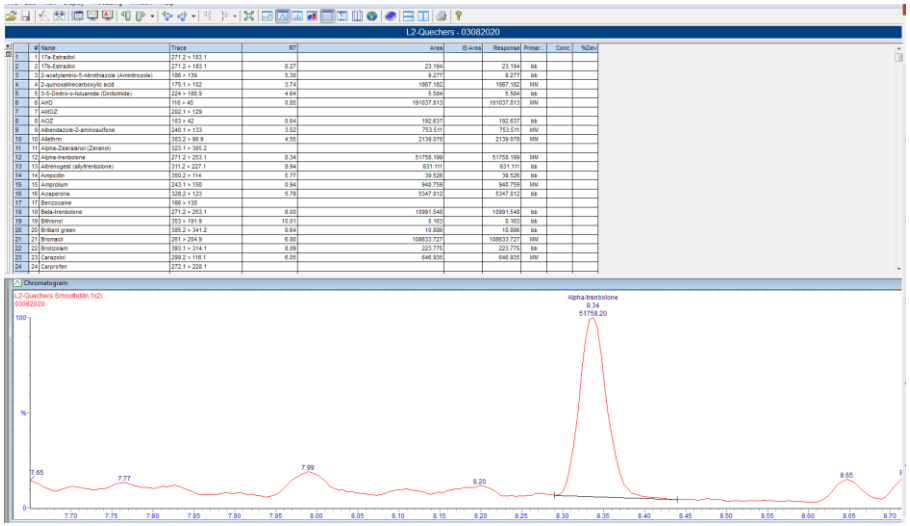
Analysis 2: LC-QTOF
PesticideScreener+ToxScreener (Bruker)
+3000 compounds

Suspect screening

Processing the data from the two screenings: comparison of experimental data with theoretical data



PesticideScreener+Toxscreener



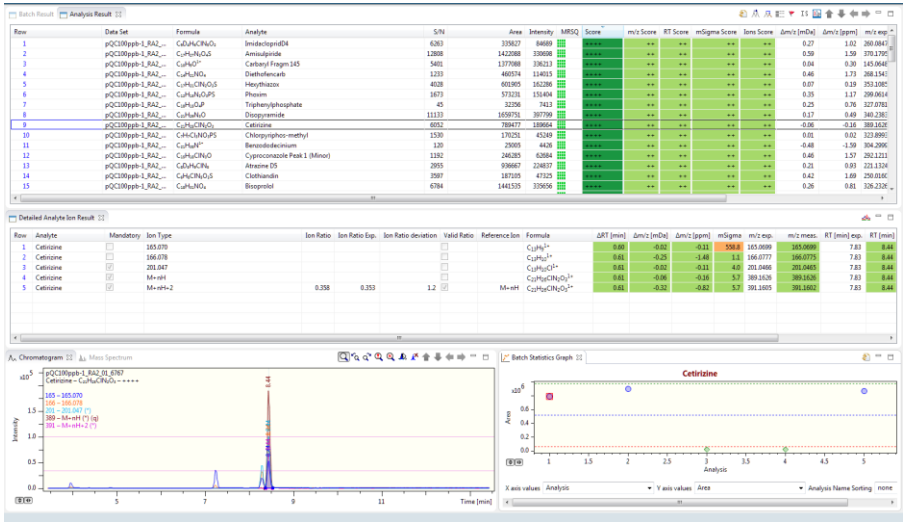
Quanpedia

- Retention time (Δt_R)
- Mass to charge ratio ($\Delta m/z$) of parent
- Isotopic pattern
- >70% fragments
- Mass to charge ratio ($\Delta m/z$) of fragments

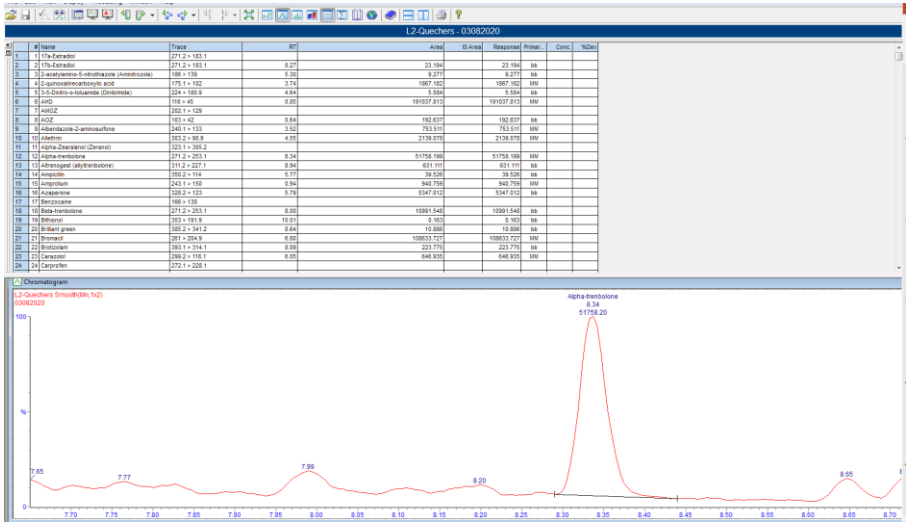
- Retention time
- MRM transition

Suspect screening

List of suspects



PesticideScreener+ToxScreener: 45 suspects



Quanpedia : 110 suspects

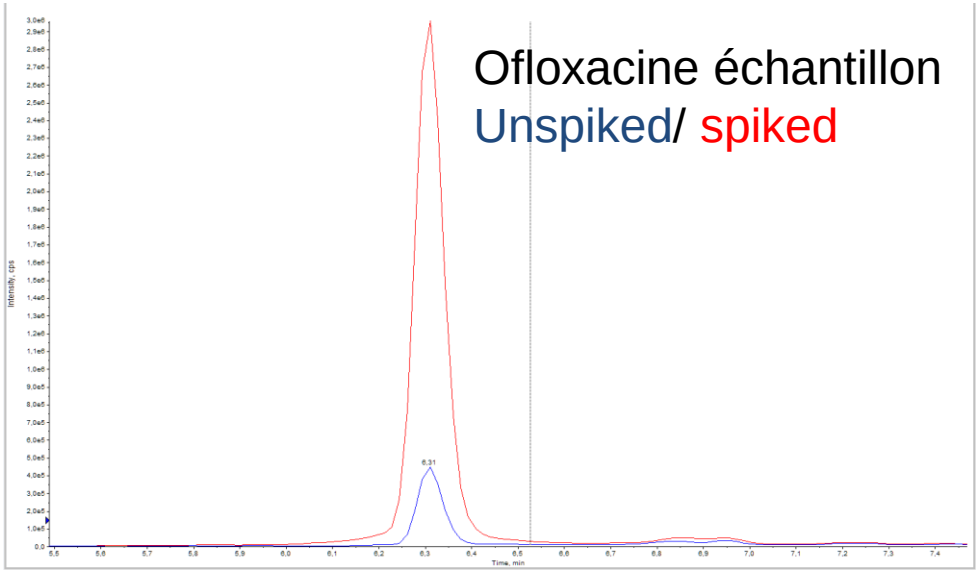
List of 30 relevant compounds for confirmation

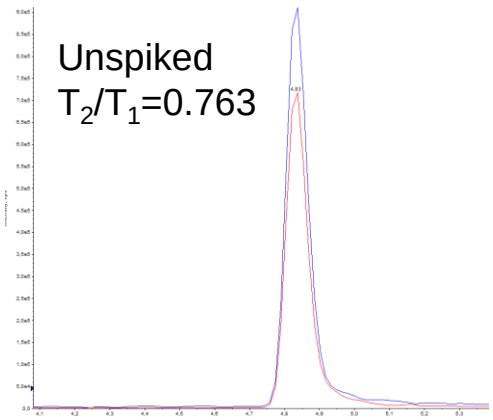
Selection criteria :

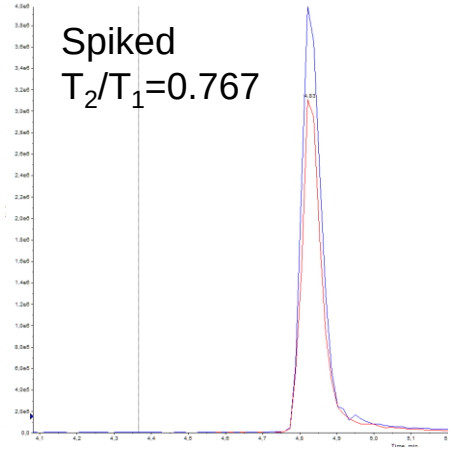
- Compounds found in sludge and at least one of the agricultural inputs
- Presence in several extracts of the same matrix
- Intensity of chromatographic peaks

Micropollutants in the environment

Confirmation by adding standards in the extracts and matching chromatographic and mass spectrum criteria



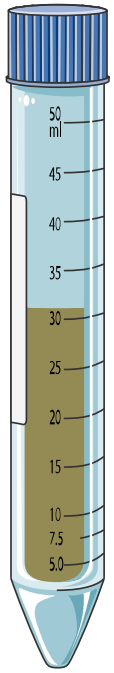




Final list

- 24 native molecules
- 10 transformation products
- 11 isotopically-labelled standards

Optimisation of a targeted analysis



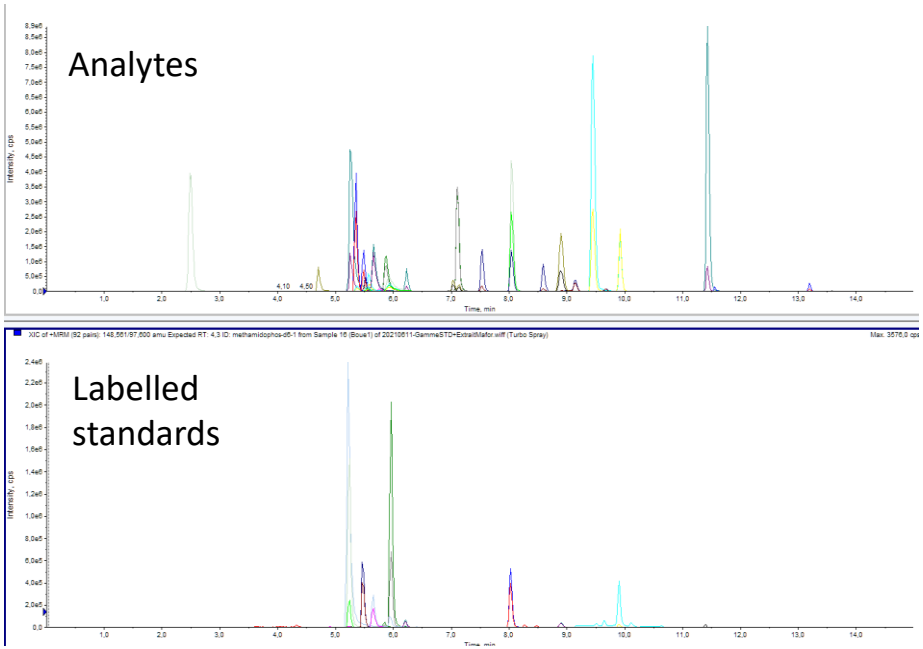
1g matrix

H_2O+ EDTA 0.1M + acetic acid ACN
5 mLHeptane
QuEChERS « Veterinary Drugs »
4g Na_2SO_4. 1g NaCl

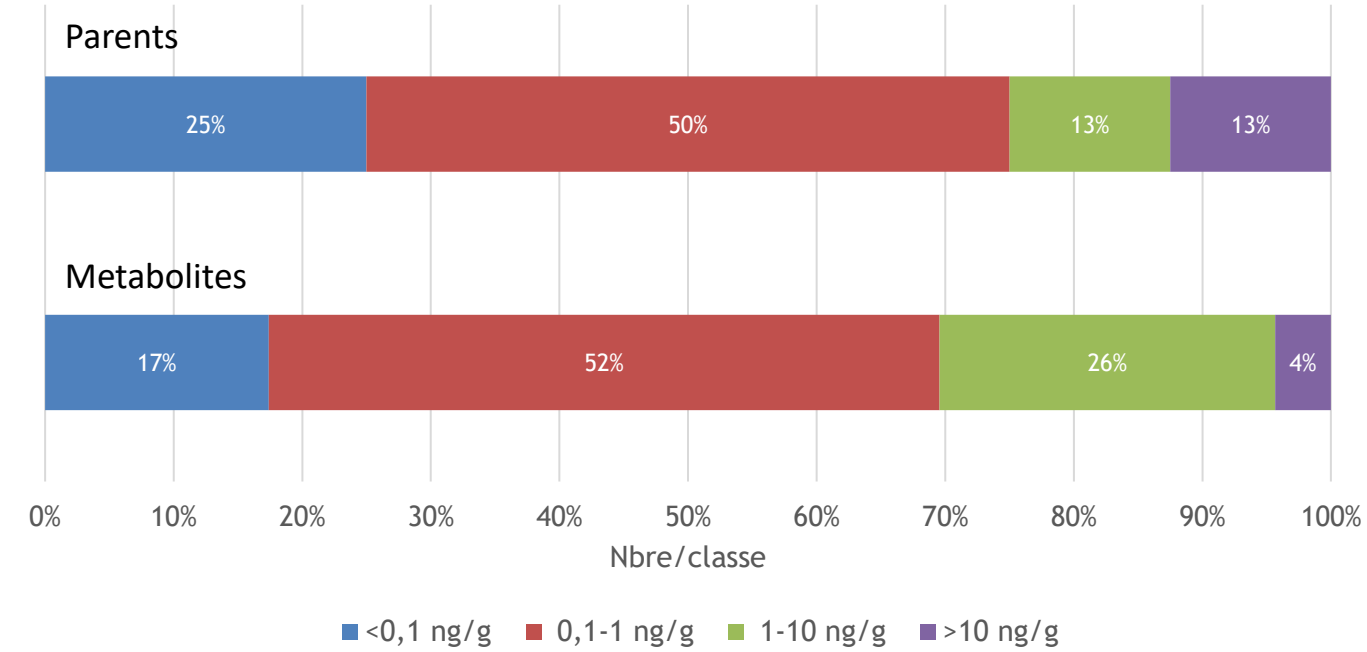
LC-MS/MS analysis (QqQ), column
HSST3 150x2.1mm; 2.5µm
A: H2O + formic acid 0.1%
B: Methanol

Extraction efficiency

23/35 : >75%
all: >45%
Labelled standards 7/10 >90%

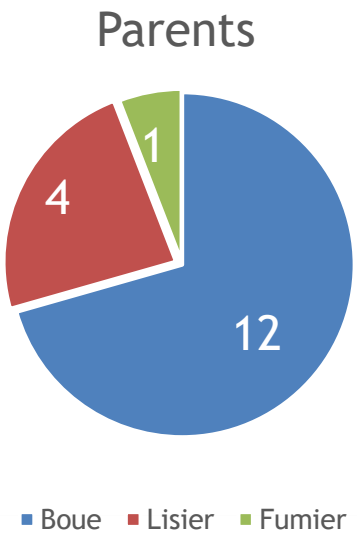


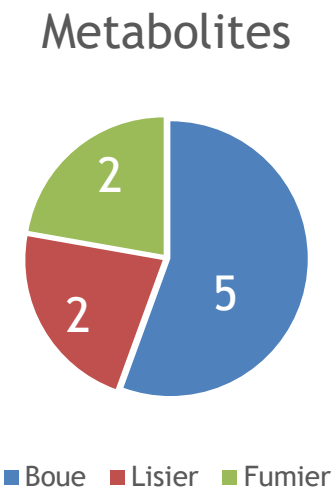
Limits of detection and quantification



90% LOQ < 10 ng/g

Consistent with trace analysis in environmental matrices

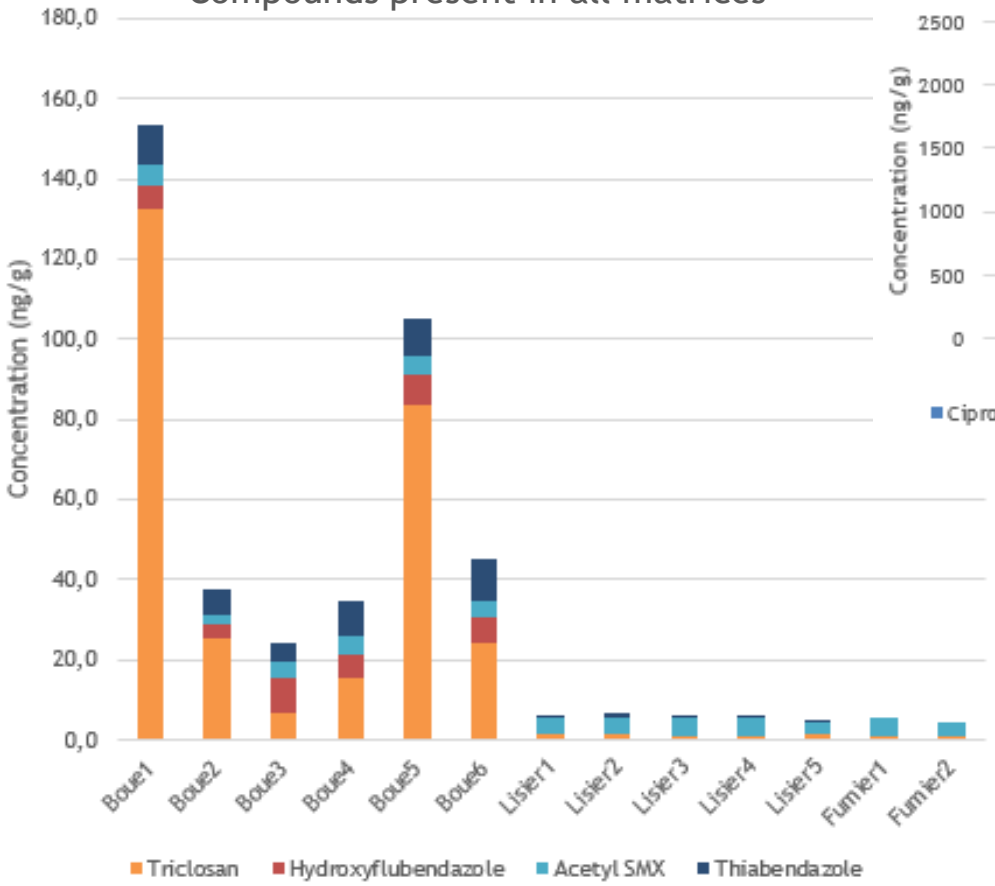




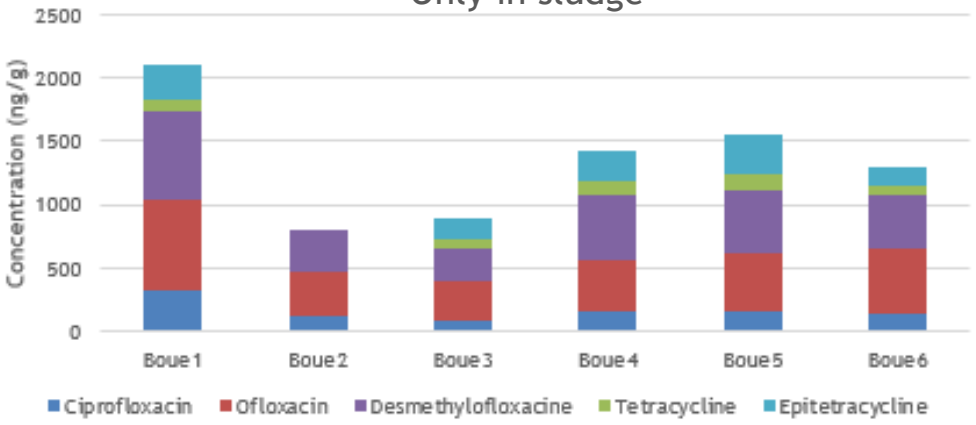
Results

Application of the targeted method to 6 sludge, 5 slurry and 2 manure

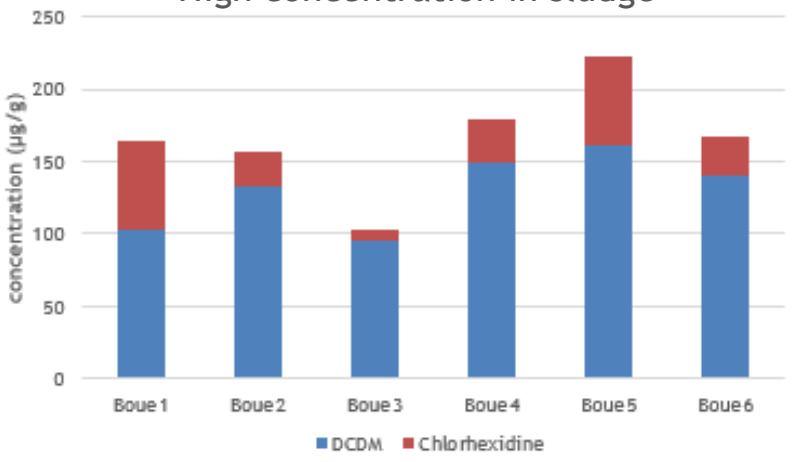
Compounds present in all matrices



Only in sludge



High concentration in sludge



Conclusions: micropollutants in complex matrices

Advantages and disadvantages of different analytical strategies

- Target: only known compounds
- Untarget: limited data base

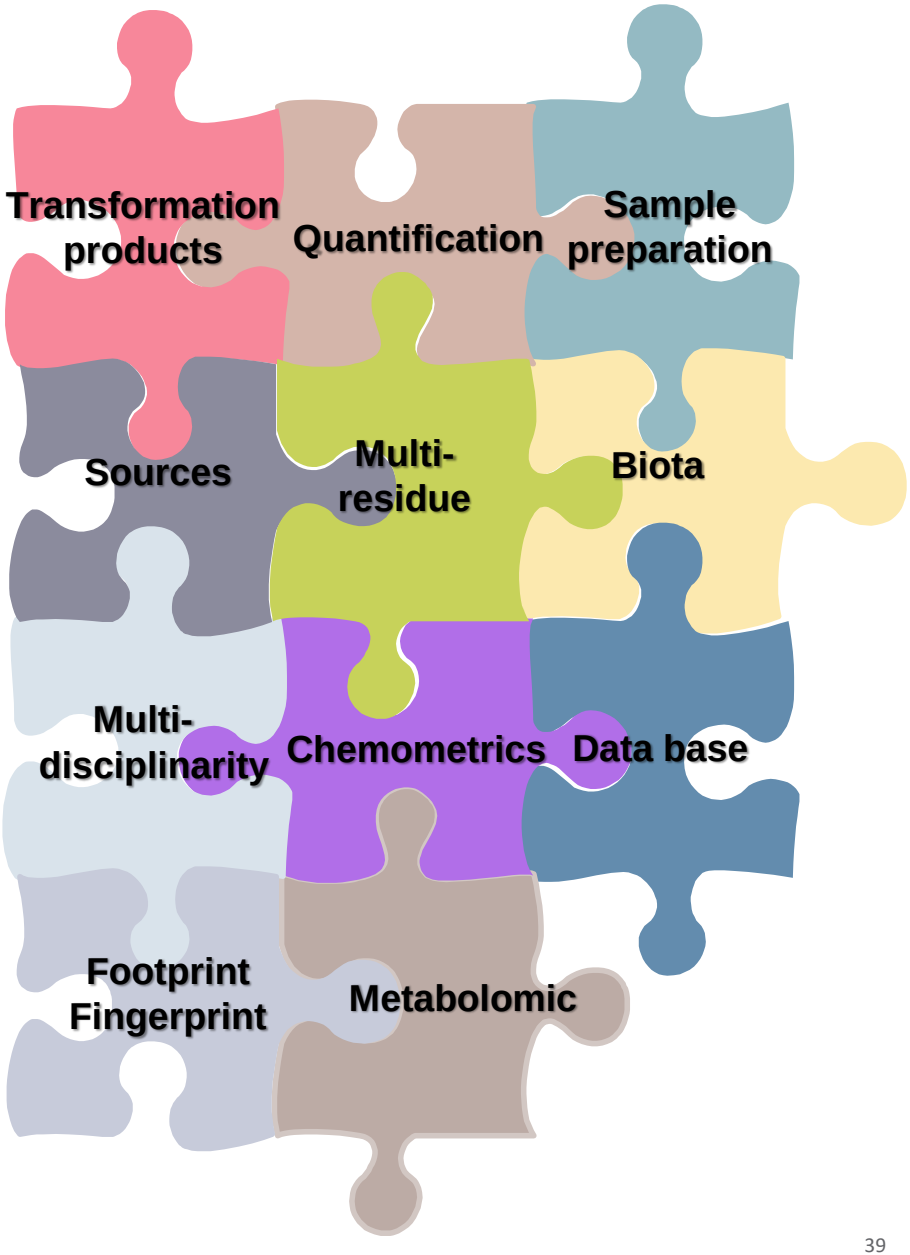
Difficulty in determining the origin of micropollutants

- Top-down
- Bottom-up

Prioritisation of contaminants

- Chemistry-driven
- Statistic-driven
- Metabolomic

....



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Site Pilote de Bellecombe

TRACES group

RISMEAU project

Fundings

Alexandre Guironnet PhD

« Développements analytiques autour du couplage chromatographie liquide-spectrométrie de masse pour la quantification des composés vétérinaires présents à l'état de traces dans des matrices complexes environnementales »

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